



Features of Heart Rate Variability Capture Regulatory Changes During Kangaroo Care in Preterm Infants

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Objective To determine whether heart rate variability (HRV) can serve as a surrogate measure to track regulatory changes during kangaroo care, a period of parental coregulation distinct from regulation within the incubator.

Study design Nurses annotated the starting and ending times of kangaroo care for 3 months. The pre-kangaroo care, during-kangaroo care, and post-kangaroo care data were retrieved in infants with at least 10 accurately annotated kangaroo care sessions. Eight HRV features (5 in the time domain and 3 in the frequency domain) were used to visually and statistically compare the pre-kangaroo care and during-kangaroo care periods. Two of these features, capturing the percentage of heart rate decelerations and the extent of heart rate decelerations, were newly developed for preterm infants.

Results A total of 191 kangaroo care sessions were investigated in 11 preterm infants. Despite clinically irrelevant changes in vital signs, 6 of the 8 HRV features (SD of normal-to-normal intervals, root mean square of the SD, percentage of consecutive normal-to-normal intervals that differ by >50 ms, SD of heart rate decelerations, high-frequency power, and low-frequency/high-frequency ratio) showed a visible and statistically significant difference ($P < .01$) between stable periods of kangaroo care and pre-kangaroo care. HRV was reduced during kangaroo care owing to a decrease in the extent of transient heart rate decelerations.

Conclusion HRV-based features may be clinically useful for capturing the dynamic changes in autonomic regulation in response to kangaroo care and other changes in environment and state. (*J Pediatr* 2017;182:92-8).

Kangaroo care refers to a period of direct skin-to-skin contact in which infants are placed in the prone position on a parent's naked chest. Kangaroo care is considered safe and has been reported to reduce morbidity and mortality, even in extremely preterm infants.^{1,2} It is associated with important physiological benefits, including promoting quiet sleep, enhancing thermoregulation, and reducing crying/fussy behavior,³ and can mitigate physiological responses to procedural pain.^{4,5} This indicates that parental coregulation is superior to regulation within the incubator environment, and that kangaroo care positively influences autonomic regulation.⁶ The ability to track and quantify any changes that occur as a result of kangaroo care may aid in detecting and establishing patterns of improved regulation in preterm infants and may offer opportunities to enhance neurodevelopmental care and homeostatic regulation.

The role of the autonomic nervous system in controlling homeostatic regulation can be evaluated by tracking cardiorespiratory variables, including heart rate (HR), respiratory rate (RR), oxygen saturation (SpO₂), and temperature. Previous studies have shown that these variables remain stable during kangaroo care,⁷ supporting the conclusion that kangaroo care is safe; however, this finding in itself does not provide insight into the underlying physiological changes triggered by kangaroo care.

The physiological phenomenon of HR variability (HRV, defined as the variation in intervals between consecutive heartbeats) can provide additional

ECG	Electrocardiography
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
LF	Low frequency
NICU	Neonatal intensive care unit
NN	Normal-to-normal
pDec	Percentage of decelerations
pNN50	Percentage of consecutive normal-to-normal intervals that differ by >50 ms
PSNS	Parasympathetic nervous system
RMSSD	Root mean square of the standard deviation
RR	Respiratory rate
SDDec	Standard deviation of deceleration
SDNN	Standard deviation of normal-to-normal
SNS	Sympathetic nervous system
SpO ₂	Oxygen saturation

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physiological insight into these regulatory changes. HRV reflects the dynamic, rapidly occurring changes in autonomic regulation caused by the primary systems controlling the HR. In addition to humoral factors, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS) can influence HR instantly, that is, from beat to beat.^{8,9} Multiple features analyzing these beat-to-beat changes have been constructed and studied in adults, with at least some consensus regarding their interpretation. For instance, the SD of the R-R peak intervals reflects overall variability, whereas differences in successive R-R intervals largely reflect PSNS activity.⁸

HRV has been less thoroughly explored in neonates, however. Moreover, the behavior of the neonatal heart, particularly in the premature neonate, is significantly different from that of the adult heart, reflecting underlying differences in autonomic regulation. This suggests that interpretations of HRV-based features in neonates may differ from those of adults.^{9,10} For example, unlike adults, neonates display a significantly larger range of variation in HR and RR and are prone to both acute tachycardia and bradycardia,^{9,11} suggesting that HRV features should account for this intrinsically different aspect of neonatal physiology.

Although kangaroo care has been shown to enhance autonomic maturation,¹² to date few studies have examined the dynamic changes in HRV during kangaroo care, notably without consensus on the findings.^{13,14} No studies have visualized changes in HRV during kangaroo care and compared it with HRV while the neonate was in the incubator.

We hypothesized that regulatory changes in preterm infants can be captured using HRV-based features. We used HRV-based features to track regulatory changes that occur as a result of kangaroo care, a period of improved regulation. We also analyzed HR, RR, SpO₂, and temperature to provide a holistic perspective on regulation.

Methods

The Máxima Medical Center has an 18-bed, level III, tertiary neonatal intensive care unit (NICU) with private rooms in which kangaroo care is practiced routinely. Parents are encouraged to perform kangaroo care for durations of 60 minutes or longer. Routine patient monitoring continues during kangaroo care, including electrocardiography (ECG), HR, RR (using impedance pneumography), SpO₂, and temperature (measured in the diaper). The patient monitors (IntelliVue MX 800; Philips, Hamburg, Germany) have the provision to save the variable data (HR, RR, SpO₂, and temperature; 1 value every minute) and the 3-lead ECG data (sampled at 125 Hz) of the past 72 hours of measurement. Notably, ECG sampled at 125 Hz has proven to be sufficient for determining HRV-based features.¹⁵ For this study, nurses annotated all kangaroo care sessions by recording the start time (placement on parental chest) and the end time (placement into the incubator) of kangarooing for each session over a 3-month period between August and October 2015.

This study was part of a comprehensive observational perinatal monitoring research program (IMPULS 1) conducted at

the Máxima Medical Center, in collaboration with Eindhoven University of Technology and Philips Research. Approval was provided by the local Ethical Committee. We selected neonates with more than 10 annotated kangaroo care sessions within the study period, to account for inpatient variability and maturational differences. This yielded a total of 220 kangaroo care sessions from 11 neonates (6 males and 5 females). We excluded kangaroo care sessions in which infants were mechanically ventilated or diagnosed with an infection, a congenital anomaly, or a severe brain pathology (periventricular leukomalacia or intraventricular hemorrhage grade III/IV). In addition, the kangaroo care sessions had to last 1 hour or longer, have data for at least 1 hour in the pre-kangaroo care and post-kangaroo care periods, and not overlap with the post-kangaroo care/pre-kangaroo care period of another kangaroo care session within the same infant. **Table 1** characterizes the patient metadata at birth (first 2 rows) and across the kangaroo care sessions in this study.

To observe regulatory changes in infants as a result of kangaroo care, we compared HRV features visually and statistically for the pre-kangaroo care and during-kangaroo care periods. For the purpose of visualization, we chose to retrieve data for the 60 minutes before kangaroo care, for the variable duration of kangaroo care, and for the 60 minutes after kangaroo care. Because the duration of kangaroo care was variable, but at least 60 minutes long, the first 30 minutes and the last 30 minutes of each kangaroo care session were retained for visualizations (**Figure 1**).

For statistical analyses of variable data (vital signs) and HRV features, stable epochs were determined in the pre-kangaroo care and during-kangaroo care periods (**Figure 1**). We determined that the first 30 minutes of the pre-kangaroo care epoch are stable, whereas in the last 30 minutes, nursing care events, such as diaper changes, occur with or without parental assistance. The period of kangaroo care itself was assumed to be stable after 15 minutes, allowing for the decay of any physiological changes arising from the transition. Therefore, the first 30 minutes of the pre-kangaroo care period were compared with the 30-minute epoch in between the 16th and 45th minutes of kangaroo care (displayed as the 76th–105th minutes

Table 1. Patient characteristics at birth and on the days corresponding to kangaroo care sessions

Characteristics	Median	25th percentile	75th percentile
Gestational age, wk	28.3	26.2	29.0
Birth weight, g	950	836	1273
Kangaroo care sessions, n	9	9	24.2
Duration of kangaroo care sessions, min	83	70	106.5
Interval between the first and the last kangaroo care sessions, d	16	10.5	30
Postmenstrual age during first kangaroo care session, d	28.6	27	29.6
Postmenstrual age for all kangaroo care sessions, d	30.1	28.7	31.1
Postnatal day for all kangaroo care sessions	18	10	24.2

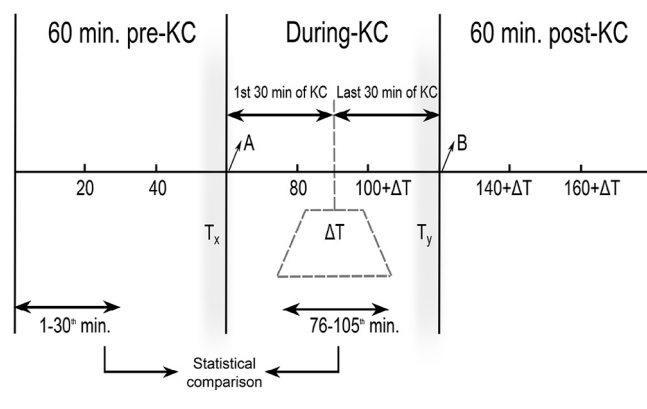


Figure 1. An illustration of the methodology. The pre-kangaroo care and post-kangaroo care sessions are 60 minutes long. The duration of kangaroo care can vary (first 30 minutes + ΔT + last 30 minutes), and thus the first 30 minutes and the last 30 minutes of kangaroo care are used for visualization. T_x and T_y represent the time to transfer the baby from the incubator to parental chest and vice versa. **A** refers to the first minute on the parent's chest; **B**, to the first minute back into the incubator. The first 30 minutes of data (pre-kangaroo care period) and the 76th to 105th minutes of data (during kangaroo care) are used as stable epochs for statistical comparisons.

of continuous data in [Figure 1](#)). The median and IQR for the mean values of the HRV features corresponding to these 30-minute epochs were calculated.

The variable values of HR, RR, SpO_2 , and temperature were sampled every minute and required no further processing. The median and interdecile ranges of the mean values of the stable 30-minute epochs from the pre-kangaroo care and during-kangaroo care periods were calculated.

We used a peak detection algorithm to detect the R-wave peaks in the ECG recordings and calculate the R-R intervals, also called normal-to-normal intervals (NN intervals), corresponding to the time intervals between successive normal heartbeats (eg, not ectopic).¹⁶ As a first step, we built normalized histograms for the concatenated pre-kangaroo care, during-kangaroo care, and post-kangaroo care periods for the purpose of visualizing the variations in NN intervals. We did this using a bin size of 20 ms; NN intervals <200 ms and >600 ms were rejected on an empirical basis. Consecutively, time domain features were obtained by calculating the SD of the NN intervals (SDNN), the root mean square of the successive differences in NN intervals (RMSSD) and the percentage of consecutive NN intervals that differed by >50 ms (pNN50) every minute using data from the previous 5 minutes. Because transient repetitive HR decelerations are potentially a sign of distress/illness,^{17,18} yet still a component that increases HRV, we developed a feature that we call percentage of decelerations (pDec), defined as the percentage of NN intervals longer than the mean NN interval of the previous 5 minutes. This feature aims at explicitly extracting variations in HRV arising from decelerations. The magnitude of decelerations (because

a HR decelerating to 120 bpm differs from a deceleration to 60 bpm) was captured in the SD of deceleration (SDDec), which is defined as the SD of all NN intervals that contribute to pDec.

We performed frequency domain analysis after resampling the NN intervals at a frequency of 20 Hz along a uniform time axis using linear interpolation and calculating the discrete Fourier transform each minute using a moving-average window of 5 minutes on detrended data. In adults, low-frequency (LF) variation reflects SNS-driven baroreceptor activity and high-frequency (HF) variation captures the vagally mediated respiratory sinus arrhythmia, resulting in a LF/HF ratio reflective of the sympathovagal balance. We calculated the LF power, HF power, and LF/HF ratio after defining the LF and HF bands as 0.04-0.15 Hz and 0.4-1.5 Hz, respectively. [Table II](#) (available at www.jpeds.com) provides an overview of the 8 HRV features that we used and their corresponding physiological interpretations in adults.

We calculated the mean \pm SEM values every minute for all HRV features using a moving-average window of 5 minutes. Because there can be differences in baseline values between different infants or between different kangaroo care sessions within the same infant (eg, as a result of maturation), we performed baseline removal by subtracting the mean value of each feature in the first 30 minutes of pre-kangaroo care from its corresponding time series.

Statistical Analyses

We compared the differences in the mean values of the variable data and the HRV features corresponding to the stable epochs of the pre-kangaroo care and during-kangaroo care periods using the 2-sided paired Wilcoxon signed-rank test. We used a left-tailed Wilcoxon rank-sum test for the concatenated pre-kangaroo care and during-kangaroo care NN intervals to test the hypothesis that the median NN intervals were higher during kangaroo care (ie, lower HR). A *P* value of .01 was considered significant.

Results

We analyzed data from the 30-minute stable epochs during kangaroo care for changes in vital signs and HRV features compared with the pre-kangaroo care incubator period, in a total of 191 kangaroo care sessions provided to 11 preterm infants. For vital signs, small changes were seen in HR and RR (*P* < .01). For the pre-kangaroo care and during-kangaroo care epochs, the median (interdecile) values for HR were 159 (146-170) and 156 (145-167), respectively, and 49 (42-62) and 47(38-61), respectively, for RR.

The normalized histograms of the NN intervals corresponding to the periods of the concatenated pre-kangaroo care, during-kangaroo care, and post-kangaroo care data are shown in [Figure 2](#) (available at www.jpeds.com). Overall, there was a greater percentage of shorter NN intervals in the pre-kangaroo care period than in the during-kangaroo care period (*P* < .001).

Table III. Values for HRV features corresponding to the stable pre-kangaroo care and during-kangaroo care periods

HRV features	Pre-kangaroo care, median (IQR)	During kangaroo care, median (IQR)	P value
SDNN, ms	42 (21-79)	29 (18-65)	.0025
RMSSD, ms	40 (15-79)	25 [12-53]	.0001
pNN50, %	2.3 (0.5-10.3)	1.3 [0.3-5.3]	.0036
pDec, %	39 (29-45)	39 [30-45]	.70
SDDec, ms	60 (26-157)	41 (19-124)	.0003
LF power, ms ²	781 (168-4486)	459 (110-2470)	.0154
HF power, ms ²	513 (64-1877)	165 (37-1311)	.0001
LF/HF ratio, normalized units	2.8 (1.6-4.9)	3.5 (1.8-6.7)	.0001

Because the transition from the incubator to the parent's chest may affect physiology, the 30-minute period between 16 and 45 minutes from the start of kangaroo care (during kangaroo care) is considered stable and is used for statistical comparisons (2-sided paired Wilcoxon signed-rank test) with the stable 30-minute period at the start of pre-kangaroo care.

Our in-depth analysis of the dynamic changes in NN intervals in response to kangaroo care using the 8 HRV features for the pre-kangaroo care period, the first 30 minutes of kangaroo care, the last 30 minutes of kangaroo care, and the post-kangaroo care period is shown in [Figure 3](#). All features responded notably to the transition from the incubator to the parent's chest and vice versa. Six of the 8 features (all but pDec and LF power) showed a significant change between the pre-kangaroo care and during-kangaroo care periods ($P < .01$) ([Table III](#)).

Discussion

In this study, we analyzed the effect of kangaroo care on features of HRV by investigating 191 kangaroo care sessions in 11 preterm infants. Six features—SDNN, RMSSD, pNN50, SDDec, HF power, and LF/HF—showed statistically significant and clearly visible differences in the during-kangaroo care period compared with the pre-kangaroo care period, and changes in vital signs were small and clinically irrelevant. Although stable vital signs during kangaroo care have been reported previously,^{1,2,7} our study used HRV to show changes in autonomic regulation as a result of kangaroo care.

Kangaroo care is a highly comfortable period for both the baby and the parent, and thus it is assumed to represent a state of improved regulation in an infant.^{1,2,4-7} The time domain measures of HRV (SDNN, RMSSD, and pNN50; [Table II](#)) decreased during kangaroo care. This was remarkable, given that in adults, a decrease in overall HRV is associated with greater cardiovascular morbidity and mortality.⁹ However, cardiorespiratory physiology in preterm infants differs greatly from that in adults; for example, preterm infants are prone to rapid and transient HR decelerations, which can lead to a flawed interpretation of overall HRV. [Figure 2](#) indirectly supports this concept; even though HR was higher in the pre-kangaroo care period (ie, a larger number of short NN intervals), there was a similar number of longer NN intervals, suggesting the presence of extensive decelerations.

Moreover, previous studies in the context of detecting neonatal sepsis have shown that abnormal HR characteristics include transient HR decelerations in addition to the normal variability.¹⁹ To capture these effects, we constructed 2 new features, the pDec and the SDDec, to study the number and extent of decelerations, along with using existing features to study overall HRV. Although the percentage of decelerations (pDec) remained remarkably similar in the during-kangaroo care and pre-kangaroo care periods, the magnitude of deceleration (SDDec) decreased during kangaroo care. This finding suggests that fewer instances of extreme, transient bradycardia result in a decrease in overall HRV (SDNN, RMSSD, and pNN50) during kangaroo care. Notably, more extreme decelerations can be seen during the stressful period corresponding to patient handling just preceding the start of kangaroo care and both periods of transfer.

We speculate that this instability leads to higher HRV as a result of an immature autonomic nervous system and a dominant SNS that tends to overshoot during autoregulation. The SNS is a slower-acting system, normally prevailed by the quicker, cholinergic, myelinated PSNS innervation.⁸ The dominance of the SNS after preterm birth has been reported previously, and is most likely a result of delayed maturation of certain PSNS branches (peaking at 31-38 weeks of gestational age and maturing beyond term).^{9,20-22} In addition, chronic exposure to stress in the NICU also might lead to a dominant SNS.²³ In neonates, chronic stress has been shown to disturb the stress axis functioning, likely causing an overactive and unstable sympathetic system.^{6,24,25} The assumption that higher levels of HRV reflect instability is supported by the fact that the SDNN, RMSSD, pNN50, and SDDec values appeared to be highest during the second one-half of the pre-kangaroo care period, a period in which patient handling frequently occurs.

We speculate that the immediate decrease in these HRV features during kangaroo care may be caused by a switch in the neural mechanisms responsible for regulating the neurobehavioral state to deal with environmental challenges, previously reported as the Porges polyvagal theory.^{26,27} Two primitive mechanisms appear to be active in the pre-kangaroo care period. These involve the dorsal branch of the vagus, responding to challenges with immobilization and freezing of regulatory systems, and the SNS, which is necessary for the “fight or flight” mode but suboptimal for subtle regulation. A third mechanism, uniquely mammalian, involves a myelinated branch of the vagus and thus enables rapid regulation of cardiac output. The decreases in SDNN, RMSSD, pNN50, and SDDec seen during kangaroo care ([Table II](#)) may represent the rapid transition from the dominant unmyelinated PSNS and the SNS to the more stable regulation offered by the myelinated vagus in response to parental coregulation. The myelinated vagus is also neuroanatomically linked to other cranial nerves that are responsible for regulating social engagement and for responding to challenges by engaging in calming/soothing behavior through social communication.²⁷ The existence of a rapid parasympathetic vagal reflex has been reported even in extremely preterm infants.²⁸ The higher time domain

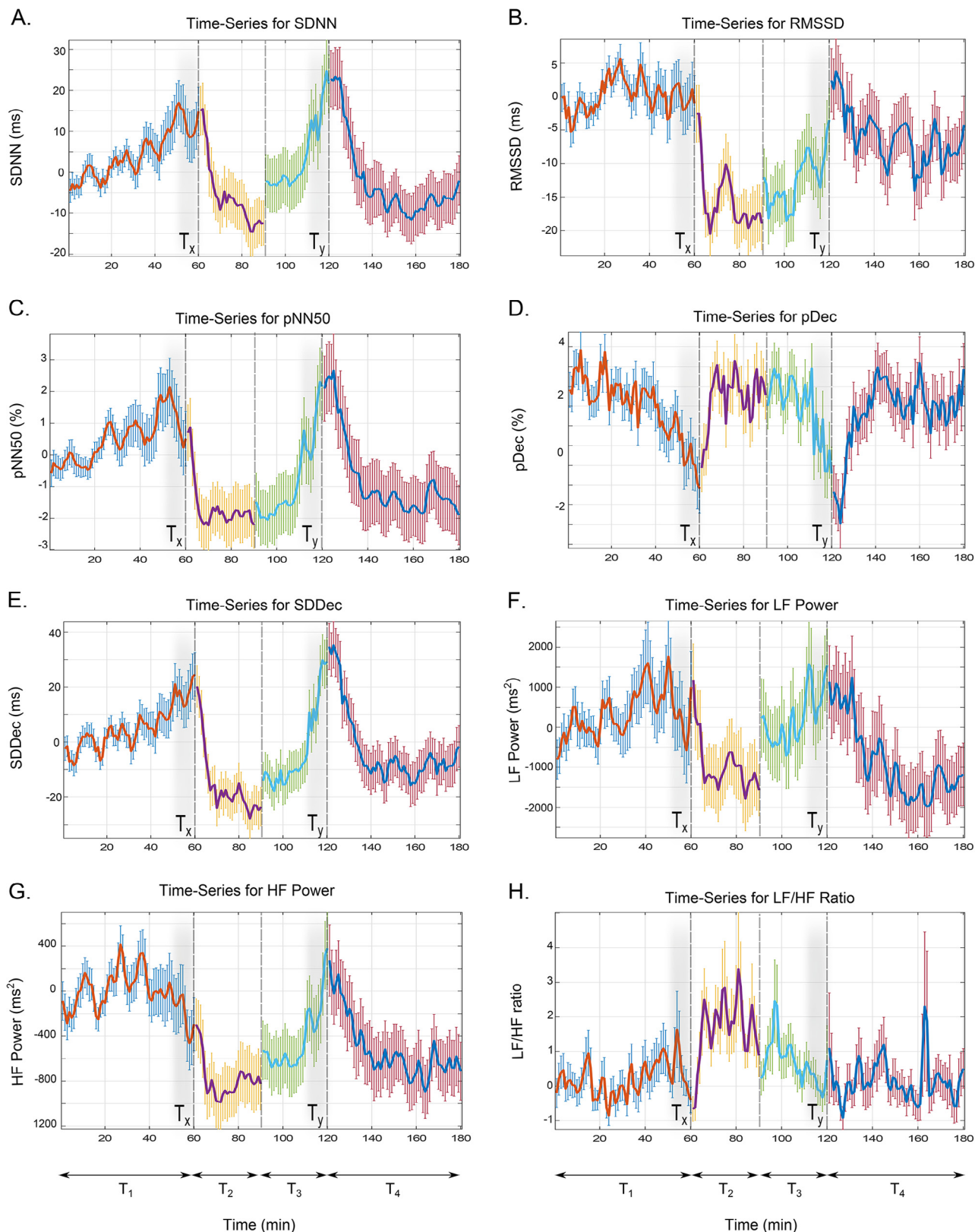


Figure 3. Time series of the 8 HRV features. Mean \pm SEM values are shown for the pre-kangaroo care period (T_1), the first 30 minutes of kangaroo care (T_2), the last 30 minutes of kangaroo care (T_3), and the post-kangaroo care period (T_4). The vertical line between T_2 and T_3 is a gap of variable length, because kangaroo care durations can exceed 60 minutes. T_x and T_y represent the periods of transfer from the incubator to the parental chest and vice versa. The y-axes represent the normalized values of HRV features. **A**, SDNN; **B**, RMSSD; **C**, pNN50; **D**, pDec; **E**, SDDec; **F**, LF power; **G**, HF power; **H**, LF/HF.

HRV values with increasing gestational age reported in multiple longitudinal studies could also be the result of a developmental shift in the balance between these mechanisms.^{29,30}

This study challenges the interpretation of HRV features in the frequency domain for preterm infants. For instance, in adults, the HF power is believed to reflect vagal activity from the myelinated branches, whereas in preterm infants, the balance between myelinated and unmyelinated vagal activity might be altered. In a study in which atropine, a PSNS blocker, was administered to 12 preterm infants aged 26–32 weeks, the LF power decreased more than the HF power.²⁸ This suggests that in preterm infants, the PSNS makes a relatively large contribution in the LF, requiring a different interpretation of LF/HF. In the present study, LF/HF increased during kangaroo care, in contrast to what would have been expected in adults. The HF power decreased significantly, likely because decelerations were less extreme and contributed fewer HF components to the total signal. These findings and the results of previous studies suggest that in preterm infants, relaxation or the sympathovagal balance is not captured by the LF/HF ratio.^{13,28,31,32} It is also possible that an increased sympathetic drive in response to kangaroo care could account for the rise in LF/HF; however, reports of increased parasympathetic behavior in response to kangaroo care suggest otherwise.^{1,2,4–7} Other research investigating HRV in preterm infants (with or without additional stimuli, like a heel prick) supports our findings in the frequency domain for routine kangaroo care.^{13,14,32–34}

This study has several limitations. Although changes in sleep stages could have contributed to the HRV changes,^{22,35} the rapid onset of HRV changes after transfers suggests otherwise, and we assume that changes in blood pressure and the change in position from the incubator to kangaroo care are more relevant covariates. In addition, mothers often start preparing for ending kangaroo care before the transfer by waking up, changing position, calling the nurse, and other actions. We believe that this is why the last period of kangaroo care (leading up to T_y; **Figure 3**) shows a dampened response. Despite these limitations, kangaroo care might be a well-controlled setting for observational studies investigating HRV in a NICU.

Another limitation is the possibility of timing errors in the nurse annotations of kangaroo care. In addition, responses to kangaroo care might depend on the sex of the infant, whether kangaroo care was performed by the mother or the father, and on the infant's age. Because HRV values vary with age (both gestational and postnatal), it is difficult to interpret them in a heterogeneous population without correction for maturation.⁸ Nonetheless, in this study each infant was his or her own control, and changes in HRV rather than absolute values were studied. Finally, infants did not contribute equal numbers of kangaroo care sessions to the study, potentially skewing the findings as a result of habituation to kangaroo care. However, even after removing the contributions of infants 1 at a time, HRV features responded to kangaroo care in a similar fashion. Moreover, the baseline removal carried out for all features inher-

ently adjusts for interpatient and inpatient differences in baseline.

Our findings suggest that it is possible to track changes in autonomic regulation across periods of kangaroo care and quantitatively establish states of increased comfort. In the future, this technique has the potential for use as a comfort tracker. Moreover, the visualization of a physiological response to kangaroo care allows nurses to promote kangaroo care and increase the involvement of family. Furthermore, as can be seen from the HRV features in **Figure 3**, there appears to be a lasting impact of kangaroo care even after the infant is moved from the parent's chest to the incubator, an important topic for future studies.

In conclusion, in this study we used features based on HRV to quantify and visualize changes in regulation as a result of kangaroo care. We developed 2 new features to independently study the contribution of transient decelerations to HRV. We found significant changes in HRV during kangaroo care relative to the pre-kangaroo care period, despite clinically insignificant changes in vital signs. ■

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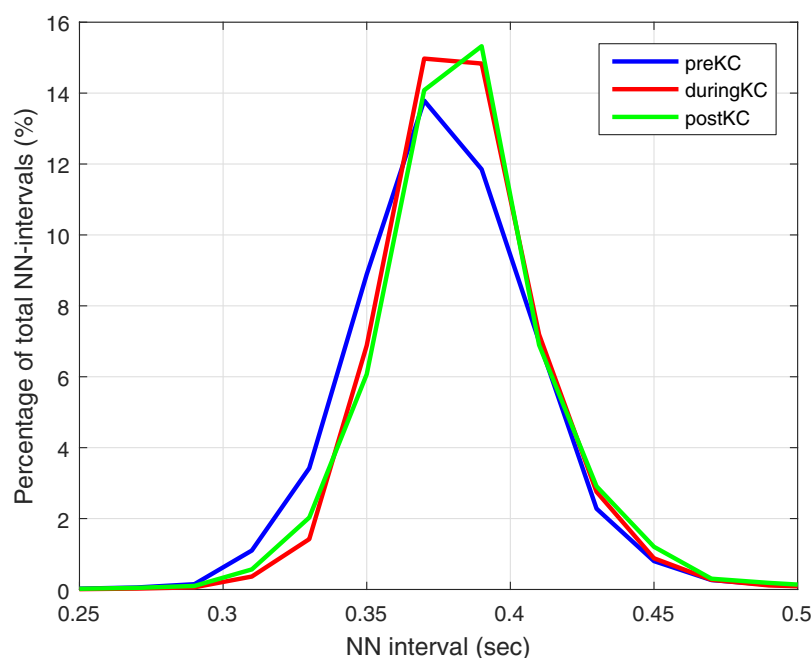


Figure 2. Normalized histograms of the pre-kangaroo care (blue), during-kangaroo care (red), and post-kangaroo care (green) NN intervals for the concatenated kangaroo care sessions. There are significantly more shorter NN intervals in the pre-kangaroo care period ($P < .001$, Wilcoxon rank-sum test), suggesting a higher HR during this period.

Table II. Overview of the HRV features used in this study

HRV features	Unit	Description	Physiological implication in adults ^{8,9}
SDNN	ms	Standard deviation of the NN intervals within a time window	Reflects overall variability and represents the beat-to-beat control mechanisms of both the SNS and the PSNS systems
RMSSD	ms	Square root of the mean of the squares of successive differences between adjacent NN intervals within a time window	Reflects HF variations, responds predominantly to changes in vagal tone, especially respiratory-associated changes
pNN50	%	Percentage of adjacent NN intervals differing by >50 ms within a time window	Reflects HF variations, responds predominantly to changes in vagal tone, especially respiratory-associated changes
LF	ms ²	Low-frequency power in a time window	Changes associated with baroreflex (mostly a sympathetic reflex)
HF	ms ²	High-frequency power in a time window	Changes associated with respiration (mostly a parasympathetic reflex)
LF/HF	n.u.	Ratio of LF to HF power	Sympathovagal balance
pDec	%	Percentage of NN intervals that are longer than the mean of NN intervals within a time window	New feature; captures HR decelerations
SDDec	ms	Standard deviation of NN intervals that are longer than the mean of NN intervals within a time window	New feature; captures the extent of HR decelerations